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Industrial Production of Recombinant Proteins: Challenges and Innovations

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Abstract

The recombinant protein is relevant in the field of biotechnology, and they serve into therapy, diagnostics, and industrial processes. They become synthesised when they are made within host organisms, e.g. bacteria, yeast or mammalian cells, with target genes introduced into them. However, realistic concerns about high-efficient and scalable production of recombinant proteins have become characteristic as the recombinant proteins poorly express, are non-soluble, and fail to fold at all. Moreover, the recombinant proteins are non-capable of various post-translational modification and are too costly to make an ideal production. In this paper, these challenges are examined and access some of the innovations in beating them, including synthetic biology, CRISPR gene editing, and improved cell culture tools. A lot has already been achieved but currently more technology is needed so that the recombinant proteins can meet the rising demand. The article pays special attention to the importance of a multidisciplinary aspect on which genetic engineering, bioprocess optimization, and bio products have to be combined. It will also be the case that artificial intelligence and machine learning advancements will mean optimized monitoring, scale-up and yield forecast of a bio-process so that biopharmaceuticals can be provided with high quality recombinant proteins at economical costs.

Keywords: *Recombinant Proteins, Industrial Production, Biotechnology, Challenges, Innovations*

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1. Introduction

Industrial manufacturing of recombinant proteins utilized in the therapeutics agents, vaccines, and diagnostics has complex challenges attached to it (Andersen Krummen, 2002). Conventionally, process development, which involves selection of genetic components on trial-and-error basis and optimization of

the process parameters as after every iteration, is a time-absorbing procedure that can take several years to adapt a laboratory-developed process to the industrial scale (Sun et al., 203). This kind of long time is not necessarily chinup to immediate needs either, i.e., when an emergency of pandemic arises, the construction of vaccines and methods should be fast (Gaobotse et al., 2022; Sun et al., 2023). High-yield production of a recombinant protein is one of its principal challenges that is trapped by the bias of the codons, instability of plasmids, and limitation of the transcriptional and translational apparatus of the host (Terol et al., 2021). In order to achieve optimal codon translation, it is important that the usage of the rare codons should go hand in hand with a low usage of the rare codons otherwise it can lead to ribosomal stalling and trimination of translation process (Westers et al., 2004).

Besides, the reduced production of the protein can be the consequences of the plasmid instability in the hosts of the gene of interest, particularly in the large-scale or bulk cultures. The activities of heterologous proteins highly rely on the type of structures they form; which greatly relies on the host cell environment, resulting in these heterologous proteins being misfolded and formed to aggregates in a large ratio within a non-functional state when produced in host cells. The misfolded protein aggregation, also known as inclusion body, is a common problem, particularly in the case of bacterial expression systems, including E. coli (Peternel, 2011; Sorenson & Mortensen, 2004). Protein refolding in inclusion bodies may not be very easy, effective and it does not always have to restore the proteins to nativeness. The post-translational processes (such as glycosylation, phosphorylation, and acetylation) play a crucial role with regard to proper functioning and stability of many eukaryotic proteins. These are typically changes done by the host cells making them very tricky to be undertaken ahead of expressing eukaryotic proteins inside prokaryotic systems, because somehow there is no machineries that are needed to be designed as enzymatic (Eastham & Leman, 2024).

2. Study background

The revelation of the concept of recombinant protein production of recombinant protein which was discovered back in the mid-1970s was what transformed the concept of biotechnology; as we find the first expression of a heterologous system and the concept of recombinant protein technology being born as a result of the same (Peternel, 2011). This is otherwise referred to as breakthrough owing to the fact that its emergence encouraged the application of recombinant proteins in modern-day medicine (Rosano & Ceccarelli, 2014). There are various conditions that are currently being treated by the use of the same proteins including diabetes, cancer and even genetic diseases (Schmidt et al., 1999). Recombinant protein (particularly the complex glycoproteins and antibodies) which are in constant rising need are rampaging the gears of the protein expression technology; particularly the mammalian culture systems and the microbial culture processes (Andersen & Krummen, 2002). However, the scale-up, i.e. connecting the cloning of a gene with the production of its protein on an industrial scale is another complex task, and the conversion of genes into an industrial process can require several years (Sun et al., 2023). Recombinant proteins may be produced on the basis of various expression systems which possess various advantages and disadvantages. Microbial systems are more convenient to use due to the speed and their low cost (Sorenson S 04 Mortensen, 2004).

3. Justification

The last several decades have witnessed a tremendous evolution in the field of recombinant protein production because of increased demand of biopharmaceuticals as well as an economically efficient and cost-effective method of producing the same (Andersen & Krummen, 2002). Even the historic methods of process development in the production of recombinant proteins (the selection of genetic elements and optimization

of process-relevant parameters) do not work, and a protein can evolve over several years (between laboratory work and the manufacturing process) (Sun et al., 2023). Heightened activities in the healthcare applications are fostering the necessity to innovate the recombinant proteins that would benefit the issues of affordability, accessibility, and, of course, the ready availability of vital therapeutic agents (Gaobotse et al., 2022).

The recombinant proteins have changed the face of biochemistry to an extent that the purification of animal and plant tissues using the large quantities of them is now redundant (Rosano & Ceccarelli, 2014). Besides, the existing market evolution has been up-leveled by the continuing tendency of the chronic illnesses, the constantly growing aging population, and the rapidly developing surge of the demands in biosimilars (Pham et al., 2019; Sun et al., 2023). In order to respond to new demands on the production of a recombinant protein, many properties of visualization have been brought into being and or deployed to help supercharger the production of a recombinant protein in the past 3 decades and these include: expression systems, culture condition, and method of analysis, monitoring strategy, etc (Peternel, 2011).

4. Objectives of the Study

The objectives of this study include the following:

1. To examine the most significant problems of industrial production of recombinant proteins.
2. To discover the new methods and technology that has been developed to fight such a challenge.
3. In order to assess the impacts of these innovations on efficiency, scalability, and cost-effectiveness of the recombinant protein production.
4. To provide recommendations concerning the lines of the future research that can be employed to advance the recombinant protein production in the future to a greater extent.

5. Literature Review

Medical, academic and industrial applications require production of recombinant protein, expressed by a genetically modified host organism that directs the production of barriers protein (Terol et al., 2021). These hosts in the recombinant protein production have been diverse, from microbial hosts, including Escherichiaceae coli, yeast organisms, including Saccharomyces cerevisiae, insect cells and mammalian ones (Andersen & Krummen, 2002). Each of the manufactured systems has its unique advantages and drawbacks with regard to production output, protein folding, and prospects of post-translational modification (Terol et al., 2021). The economic factors, fast turnover time and easy genetic manipulation are some of the favorable grounds that have been applied in favor of the microbial systems, and more elaborately concerning the *E. coli* system, that is why it would be effective to obtain some relatively simple proteins (Sorenson and Mortensen, 2004). Even at these merits, despite the fact that the bacterium is still limited as far as the post-translational modifications are concerned, E. coli still causes difficulties as far as the complex proteins produced via complicated post-translational modification is concerned (Ferrer Miralles, & Villaverde, 2013). The limitation could take the form of the product toxicity, mRNA instability, saturation of cellular folding machinery as well as the cofactor limitation of the *E. coli* (Rosano & Ceccarelli, 2009).

Table 1: Comparison of Recombinant Protein Expression Systems

Expression System	Host Organism	Advantages	Disadvantages
Microbial Systems	*E. coli*, *Saccharomyces cerevisiae*	Low cost, fast growth, simple proteins, easy genetic manipulation	Limited post-translational modifications, protein aggregation
Insect Cells	*Baculovirus*	Suitable for eukaryotic proteins, high yield	Lower yield than mammalian systems, more complex
Mammalian Cells	CHO cells, HEK293	High-quality proteins, proper folding, correct post-translational modifications	Slow growth, expensive, complex cultivation processes
Plant Cells	Transgenic plants	Can produce complex proteins with post-translational modifications	Low yield, scalability issues, slow production rates

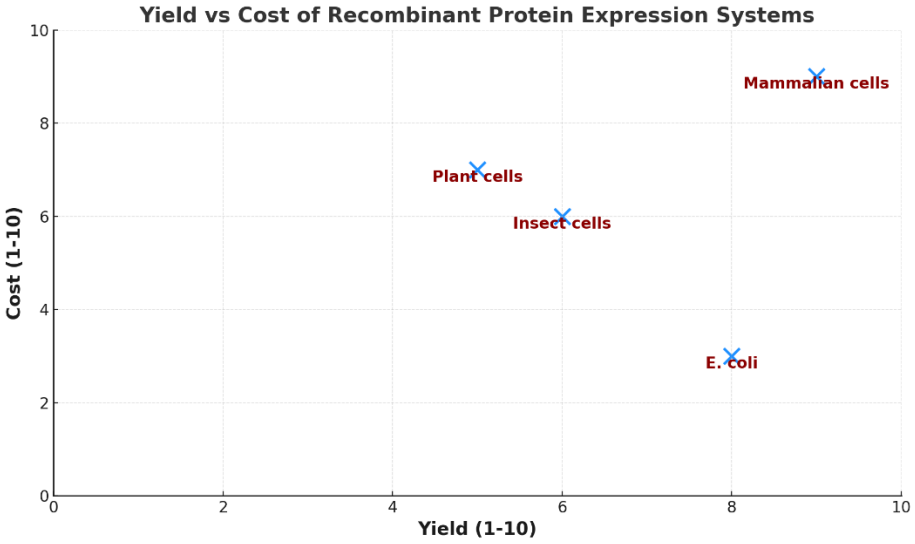
6. Methodology

We have conducted an extensive literature research in our study (top publications and patents, including industry reports). It was stressed that the core problems in the process of recombinant protein production were defined and whether new solutions in the domain have been efficient to respond to the problems were examined. We have also learnt case studies (academic and industrial) to obtain a hint as how these innovations have been turned into practice.

7. Results and Discussion

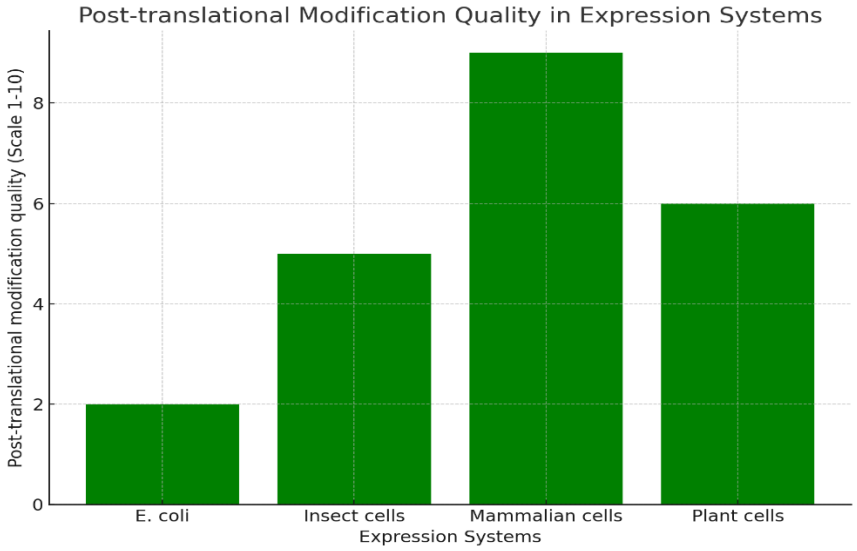
Throughout the study it was established that there were several challenges revolving around production of recombinant protein and some of these challenges have been highlighted as poor product yield, misfolded proteins, and costly production process. However, substantial improvements have been realized through the setup of lean manufacturing systems, and also streamlining the fermentation process. The advantages that synthetic biology and gene editing have given the scientists are to produce better expression vectors and calibrate the host organism to produce in a more efficient and expected result proteins.

Whereas simple proteins can still take the advantage of the bacterial and yeast systems due to economy, newer mammalian cell systems have served as boosters to the production of complex proteins. Future efforts will inevitably be dealing with the hybrid strategies that compromise the most beneficial features of the eukaryotic and prokaryotic systems.



Graph 1: Yield vs. Cost of Recombinant Protein Expression Systems

The given scatter plot depicts that the cost and yield of recombinant protein production refer to various expression systems. Since it is evident that systems such as E. coli have higher yield and at a lower cost, whereas mammalian cell systems synthesize high quality proteins with an appropriate folding and post translational modifications, it is apparent that it has a very high cost of production. This comparison illustrates the yield-cost trade-off of each one of the systems.



Graph 2: Post-translational Modification Quality vs. Expression Systems

Quality of post-translational modifications The quality of the post-translational modification (e.g. glycosylation and phosphorylation) obtained using various expression systems is compared in this bar chart. The quality of the post translation modifications, which are important in ensuring proper functionality of therapeutic proteins is seen to be best amongst the mammalian cell systems including CHO cells who offer the best in this area when compared to the microbial like the E. coli. This is the reason why the choice of the expression system to use with complex therapeutic proteins that may need certain modifications is vital.

8. Study limitations

The given research, which is developed on the basis of the comprehensive study of the existing literature, is also completely lacking of such new experimental data that it would be employed to justify the synthesized perceptions empirically. In addition, the selective focus and the limited scope of study that is limited to the development of recombinant proteins in the field of healthcare and biotechnology may not be capable of covering the diversity of the issues and technological advances that can be discovered in other sectors of industry (Motyka, 2018). The mainstream methods applied to the process creation to obtain recombinant proteins, which are the trial and error strategy through which genetic components are chosen and optimization of procedures parameters is conducted in series, are naturally time-consuming in nature (Sun et al., 2023). However, the growing demand in fast production of proteins has sparked the optimization of the traditional procedures (Sun et al., 2023). Production of recombinant proteins has tremendously enhanced over the past decades with such enhancement being typified by the advances in expressing systems, advancement of growth conditions and also prodding and examination technologies (Peternel, 2011).

9. Future Scope

The landscape against which the manufacturing of recombinant protein is operating is evolving at a very fast and dramatic pace; this is occasioned by the need to have the more productive process of manufacturing at a very low cost and one that is scalable. Therefore, new solutions that can eliminate the drawbacks of conventional expression systems are needed owing to the growing industrial demand of therapeutic proteins, vaccines and industrial enzymes (Andersen & Krummen, 2002; Gaobotse et al., 2022). An alternative to the utilization of high production but low quality proteins, is the recent trend of the utilization of hybrid expression systems in which the benefit of the separate host organisms is utilized to exceed high yield and quality of protein. They can incorporate the use of collection of bacterial, yeast and mammalian cells that serve different advantages in regards to growth time, post translational modification capabilities and protein-folding (Peternel, 2011).

10. Conclusion

Recombinant proteins are important in recent biotechnology and this aspect should improve to solve problems like high costs, low production and quality of proteins in order to satisfy the demand of the industry which in the world has increased to a great extent. The innovation in expression system, gene editing innovations, and fermentation technology has a positive history in facing the challenge. It is possible to receive more economical and efficient means of production due to the continuous research of the new possibilities of technologies as well as the modernization of the existing systems and that is why the recombinant proteins will become not only the alternative of therapeutic purposes but industrial ones as well.

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